FURTHER STUDIES ON "BLACKHEAD" IN TURKEYS, WITH SPECIAL REFERENCE TO TRANSMISSION BY INOCULATION

E. E. Tyzzer and M. Fabyan

From Harvard University Medical School, Boston

Although the parasite causing the disease in turkeys popularly known as "blackhead" was discovered many years ago, the mode of its transmission has not been satisfactorily demonstrated. Several distinct problems are involved in this question of transmission: (1) the source of the parasite; (2) the route by which it enters the body of the turkey, and (3) its pathogenic properties or virulence.

Whether the parasite of blackhead, Histomonas meleagridis, occurs only in association with the disease, and is thus to be regarded as uniformly pathogenic, or is widely prevalent in the cecal contents of normal turkeys, and only invades the tissues under conditions which lower the host's resistance, has, up to the present, been regarded as a more or less open question. The possibility that this organism, while pathogenic for the turkey, may occur commonly in the intestinal tract of other species, such as the common fowl in which it may rarely produce lesions, should also be considered. The determination of any of these various points concerned in the transmission of blackhead may prove of practical importance in the rearing of turkeys.

Source.—Considerable information is already available as to the sources of the infection. That the disease may be acquired by the exposure of normal turkeys to those which have shown evidence of infection, has been experimentally demonstrated by Theobald Smith,[1] and Tyzzer.[2] Smith[3] concludes that turkeys which have passed through an attack are more dangerous as sources of infection than are those showing symptoms of active disease. Blackhead occurs chiefly in young turkeys during the summer months, and acute cases are often lacking throughout the greater portion of the year. It is obvious, therefore, that other sources of infection exist. We are not warranted, however, in assuming from Smith's results that the acute

Received for publication June 1, 1920.

[1] Jour. Med. Res., 1915, 33, p. 243.
[2] Jour. Med. Res., 1919, 40, p. 1.
[3] Jour. Exper. Med., 1917, 25, p. 405; 1920, 31, p. 633.

case is negligible as a source of infection, for mere exposure to infected turkeys — probably on account of the numerous uncontrolled factors in the procedure — has furnished notably variable results. An instance of the transmission of the disease from turkeys with acute cases to a normal turkey will be presented below. It is not known how long the parasite may persist in turkeys after recovery from blackhead, but it is to be suspected that such birds may serve as "carriers" and transmit the disease to the young of the succeeding season.

The common fowl, although it rarely shows lesions characteristic of blackhead, should also be regarded as a probable source of the infection. Smith has recorded an outbreak of blackhead in a brood of young turkeys not exposed to other turkeys, but confined near a hennery. The appearance of the disease in two lots of young turkeys following exposure to a flock of common fowls in which the disease was known to have been present earlier in the season, has been noted by Tyzzer — although the conditions of the experiment were not as carefully controlled as desired. Blackhead has also been observed by this author in a brood of young turkeys on a small farm where there were hens but no other turkeys. The view is frequently expressed that young turkeys should be raised on new, uncontaminated soil, but there is at present no reliable data available to show how long the virus remains alive outside the body.

Mode of Entrance of Parasite.—The lesions of blackhead as it occurs naturally are usually confined to the ceca, liver and contiguous surfaces to which the lesions may extend. Since the parasite evidently first invades the cecal wall and later reaches the liver by way of the portal system, it appears probable that it enters through the alimentary tract by the ingestion of contaminated food or water.

There is apparently a widespread belief that damp surroundings or wettings are fatal to young turkeys, and that wet seasons are especially unfavorable. The following extract from "Josselyn's Voyages— An Account of Two Voyages to New England, by John Josselyn, Gent., London — Printed for Giles Widdows at the Green Dragon in St. Paul's Churchyard 1674"— indicates the antiquity of this belief and also suggests the possible occurrence of blackhead in early colonial days. On page 99 appears this passage:

"The turkie, which is in New-England a very large Bird, they breed twice or thrice in a year, if you would preserve the young chickens alive, you must give them no water, for if they come to have their fill of water they will drop away strangely, and you will never be able to rear any of them: they are

excellent meat, especially a Turkie-Capon beyond that, for which eight shillings was given, their eggs are very wholesome and restore decayed nature exceedingly. But the French say they breed the Leprosie: the Indesses make coats of Turkie-feathers woven for their children.

The Partridge is larger than ours, white flesht, but very dry. They are indeed a sort of Partridges called Grouses, etc."

An outbreak of blackhead in turkeys is often attributed to a storm or wet weather immediately preceding the appearance of symptoms, but this is disproved by experiments showing that an incubation period of at least ten or twelve days is necessary before symptoms appear. That cold, damp surroundings and an occasional wetting will not of themselves produce blackhead has been amply demonstrated by the authors' experience in rearing turkeys without loss from disease under such conditions. Normal turkeys acquire it only when the virus of the disease is available; as for example, by exposure to diseased or convalescing birds or to "carriers." Thus, although the parasite of blackhead is undoubtedly widely distributed, it is probably not ubiquitous and is evidently not present in normal young turkeys. Whether dampness and wet weather favor infection by lowering the resistance of the host or by favoring the transmission of the parasite has not yet been demonstrated. If the parasite proves to be readily destroyed by drying, the greater frequency of infection in wet weather is explained by the favorable conditions for its tranmission, rather than by the lowering of the resistance of the turkeys.

The feeding of blackhead lesions to normal turkeys has frequently resulted negatively. The question of the presence of living virus in such material, the possibility either of its passage through the intestinal tract without entering the ceca, or its destruction by normal digestion, or that some special mechanism is required for it to penetrate the mucosa of the ceca, all furnish problems for speculation, as nothing is definitely known concerning these points. The exposure of normal to infected turkeys is also productive of variable results, and on account of its uncertainty, cannot be relied on as an experimental procedure.

Pathogenicity.—Until the disease can be produced at will, it is difficult to draw any definite conclusions as to the presence of infective virus, its pathogenicity, or to determine the susceptibility of various species suspected of being sources of infection. When it is possible to produce the disease constantly with a given virus, the properties of the latter may be determined, such as its resistance to various external conditions, as well as to various drugs therapeutically administered to

the infected host. Such experiments should also throw light on the question of the incubation period, the possibility of unrecognized attacks, latent infection, "carriers," etc., and also on the pathology of blackhead.

Experiments which were carried out during the summer and autumn of 1919, have served primarily to demonstrate the uniform susceptibility of turkeys, and the relative nonsusceptibility of a number of other species of birds and of laboratory mammals to the inoculated disease. In addition to this: (a) the infectiousness of the inoculated disease has been demonstrated; (b) an attempt has been made to transmit the disease through the agency of "blow flies"; (c) confirmatory evidence relating to the common fowl as a source of the disease has been obtained; and (d) attempts have been made by the administration both of tartar emetic and of quinin to destroy the parasite in the tissues of the inoculated turkey.

The virus employed in the inoculation experiments was derived primarily from the liver lesions of freshly killed young turkeys in the acute stage of the disease, but was subsequently taken from the lesions of inoculated birds. Turkeys, chickens, pigeons, rabbits, guinea-pigs and mice were inoculated. The method of inoculation usually employed was to implant, by means of an inoculation trocar, several milligrams of material obtained from active lesions, either beneath the skin or in the muscle. The turkeys were hatched in the laboratory and reared in an enclosure outside; young chickens, and three quarter grown White Leghorns were obtained from eggs hatched in an incubator at the laboratory, and large Rhode Island Red chickens were purchased; pigeons were obtained in the open market and with two exceptions were of the common variety; and the mammals employed were all laboratory stock animals. The turkeys were reared under the following conditions.

Eggs, obtained on May 31, from a flock in which blackhead had occurred during the previous season, were incubated under common hens until they commenced to hatch, when they were transferred to an incubator. Of the 24 eggs incubated, 3 were sterile, 1 was crushed near the time of hatching, and 20 young turkeys were hatched on June 27. One with a large unabsorbed yolk sac died several days later. Within 48 hours after hatching, these turkeys were placed outdoors in a small brooder house opening out into a small wire enclosure. When about a fortnight old they were allowed the run of the yard in which turkeys had been reared and exposed to blackhead the previous summer.

On August 9, forty-three days after hatching, 2 of the largest of the lot were found dead, evidently suffocated as the result of the entire flock crowding under a sloping board platform during an unusually cold night. Neither of these 2 birds showed any lesions of the internal organs. Three days later the flock suffered another loss, one turkey having been taken and another fatally injured by a cat. In order to avoid further depredations, the turkeys were subsequently confined daily in the brooder house and attached wire enclosure between 5 p. m. and 8 a. m. until they were 2 months old, when a larger cat-proof enclosure was constructed for them to stay in during the night. For 8 hours during the day they were allowed the run of the entire yard. The space available for forage amounted to about 15 sq. yds. for each turkey. With confinement each night in the brooder house, together with continual wet weather, the feathers became badly soiled and the feeding habits became much less cleanly than those of turkeys having a free range. Their appearance improved as soon as the larger enclosure was provided and a satisfactory rate of growth was maintained in these somewhat limited quarters (see chart 1). The diet consisted of mixed grain, grit, sour milk, and an abundance of green forage, chiefly dandelion. Under these conditions 15 young turkeys were reared for the experiments reported below.

On account of the number and similarity of the experiments, and of the results obtained, the detailed protocols of only a few illustrative cases will be presented. The results of the inoculations will be discussed with reference to the different species employed, while the more important data obtained in the various experiments will be furnished in part by a chart showing the growth curves of the inoculated turkeys and in part by tables.

INOCULATED BLACKHEAD OR HISTOMONIASIS

Turkey.—The subcutaneous inoculation of young turkeys with bits of fresh liver lesion from an acute case of blackhead, has produced a characteristic and invariably fatal disease. Seven turkeys inoculated in this manner on one occasion, and others inoculated successively later on, have either succumbed to the disease or were in a dying condition when killed. The following case will illustrate the course of the inoculated form of the disease:

TURKEY 19-1.—Aug. 14, 1919: Weight, 550 gm.; inoculated subcutaneously in the left breast with a bit of liver lesion from Turkey C. P. S. 19.48 (obtained from a small flock several of which had already succumbed to blackhead).

Aug. 22: Weight, 690 gm.; an indurated mass, elevated, with well defined border, but not more than 1 cm. in its greatest diameter, was apparent at the site of inoculation. Several yellowish opaque areas in its substance were apparent through the skin.

Aug. 23: Rectal temperature at 12:30 p. m. 108.2 F.

Aug. 25: Inactive and rather weak. The breast showed a rather diffuse swelling, 2 x 3 cm. in diameter, with the muscle extensively involved. (Sulphur colored droppings noted in yard.)

Aug. 26: The lesion showed a more definite border and measured 3.5 cm. in length. The muscle appeared to be involved. The skin was intact and only slightly adherent to the surface of the lesion.

Aug. 29: Weight, 550 gm.; the lesion was roughly about one-half the size of a hen's egg and somewhate flattened; extreme weakness. Coughing had been noted among the inoculated turkeys for several days. (Since involvement of the lungs was not anticipated in the turkeys first inoculated, respiratory symptoms were not at first recorded for each individual turkey.)

Aug. 30: Found dead. Weight, 520 gm.; the subcutaneous mass measured 6 x 4 x 3 cm., and presented a central necrotic portion measuring 2 x 1.5 cm., of a firm dry texture and of a color ranging from yellowish to dull pink. Peripherally the mass consisted of grayish somewhat translucent tissue. The adherent breast muscle was reddened to a variable extent and was of soft consistence. The right lung contained a nodule (1 cm.) of firm, yellow tinged material surrounded by reddened edematous, lung tissue. There were also several ill-defined grayish lesions. The left lung showed more or less confluent, indefinite, grayish lesions, of soft consistence and without evidence of necrosis. The liver presented 6 small rounded lesions (3-4 mm.) with sharply defined borders, color pinkish, opalescent, mixed with yellow. Heart, kidneys and spleen appeared normal.

Histology.—Stained sections of the subcutaneous lesion showed an irregular, sharply defined, necrotic portion surrounded by a layer of inflammatory tissue, in places 1 cm. or more in thickness, for the most part distended with innumerable parasites. The necrotic portion was composed in part of dense appearing hyaline substance, and in part, of a reticulum of fibrinoid material, the interstices of which were filled with polymorphonuclear leukocytes, moderate numbers of other cells and parasites. The denser necrotic material was surrounded by a layer of giant cells. The peripherally situated inflammatory tissue showed dilatation of the blood vessels. There was also an infiltration with cells chiefly endothelial in type, with occasional polymorphonuclear leukocytes and small collections of lymphoid cells. The parasites were either free or within cells, the latter assuming the character of giant cells in the older portions of the lesions. At the periphery of the lesion the muscle fibers were widely separated with parasites and infiltrating cells. Many of the muscle fibers appeared swollen, stained faintly, and in some instances were disintegrating. The arrangement of the parasites indicated that they had replaced the muscle fibers.

In the sections of lung, the lesions showed the essential characteristics of the subcutaneous lesion. The most prominent feature was the infiltration of the tissue with large numbers of parasites. There was a small necrotic portion and at the periphery a well defined zone of cell infiltration. The interlobular portion of the lung was first invaded by the parasites leaving the infundibular portions relatively free. The air spaces of the affected areas showed large numbers of cells containing parasites and a small amount of serofibrinous exudate.

The liver lesions showed microscopically necrotic parenchyma intermingled with collections of organisms, and resembled those of spontaneous blackhead. Numerous giant cells were present.

The disease was transmitted by inoculation from turkey to turkey through 6 transfers, and was propagated in this manner from Aug. 14 to Nov. 7, a period of 85 days, without any diminution in its virulence. Its general character was maintained through the successive transfers.

Although it showed a remarkable regularity in its course, in some cases the respiratory symptoms were more marked than in others, and one showed symptoms suggesting involvement of the nervous system.

The primary lesion develops as the result of the multiplication of parasites at the site of inoculation. The implant is readily distinguished from the first as a small flattened nodule palpable through the skin, and usually shows no appreciable increase in size up to the sixth day. At this time or soon after, there is a well defined swelling of the tissues around the nodule which becomes indurated and subsequently increases rather rapidly in size. There is no tendency to ulceration and the skin has remained intact in every case, although these subcutaneous lesions have attained great size — in one instance measuring 8x4x1.5 cm. The nodule, which is never more than slightly adherent to the skin, is often firmly adherent to the breast muscle and may involve it to a variable extent as the lesion enlarges. It has an even rounded contour and is invariably flattened (see fig. 1). Its outer surface presents a centrally situated depressed area of opaque, dull yellowish or grayish color, and bulging, rounded border. The nodule is of firm consistence and on section shows a central, apparently necrotic, portion composed of dry caseous material varying in color from grayish to dull pink or yellowish (fig. 2). Around this, except for the external depressed area, is a layer of opalescent, slightly pinkish or reddish tissue which is thickest at the border of the nodule. Occasionally, there is a more or less, bright yellow, gelatinous exudate infiltrating the surrounding tissues.

In several of the inoculated turkeys the subcutaneous lesions have failed to attain great size and at the time of death appeared to consist of little more than a hard, flattened mass of necrotic tissue. It became evident in the course of the observations that the development of the subcutaneous lesion had no appreciable effect on the growth and health of the turkey, the progress of the disease depending on the involvement of vital organs.

Histologically, the primary lesion shows a central necrotic portion with irregular extensions into the surrounding tissue. In this are necrotic muscle fibers and obliterated blood vessels, but the tissues are, for the most part, replaced with a reticulum of fibrinoid material the interstices of which are filled with polymorphonuclear leukocytes and parasites, many of which are degenerated. The necrotic portion frequently shows concentric zones indicating its increase from time to

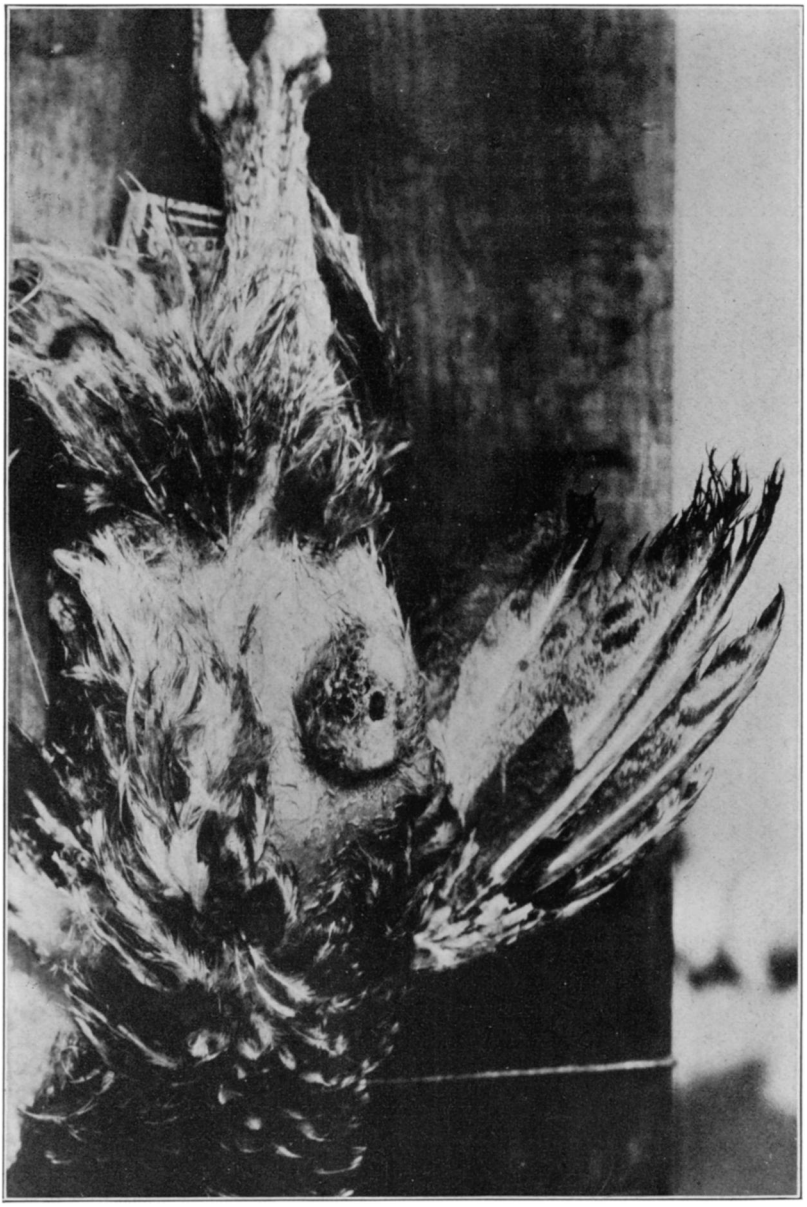

Fig. 1.—Subcutaneous lesion of turkey 19-7, which died 18 days after inoculation; × ½.

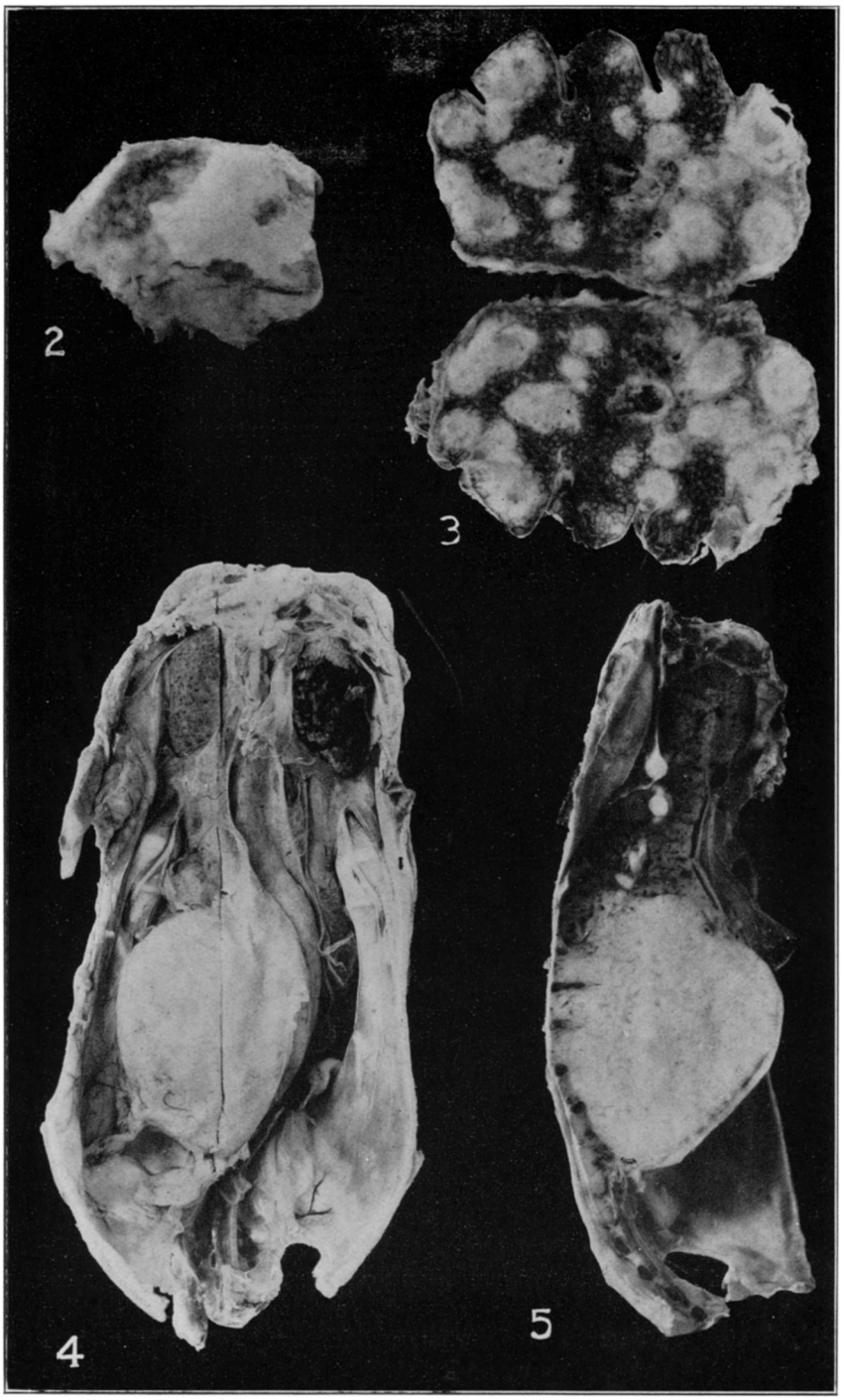

Fig. 2.—Cut surface seen on vertical section of a portion of the subcutaneous lesion of turkey 19-1. Central necrotic portion represented by light area above and to right, infiltrated peripheral zone to left of this and invaded breast muscle below; × 1½.

Fig. 3.—Surfaces of sectioned lung of turkey 19-10 killed on showing dyspnea 13 days after inoculation. The whitish areas represent focal lesions. The prominence of the lobular markings is due to a general perivascular and peri-infundibular infiltration; × 1.

Fig. 4.—Ventral aspect of dissection showing a large lesion of the right kidney of turkey 19-2, killed 25 days after inoculation. The surface of the uninvolved anterior extremity of the kidney is apparent above; × 1.

Fig. 5.—Cut surface of specimen shown in Fig. 4, section longitudinally through right kidney. The lesion appears as a large nodule molded on one side to the pelvic bones and bulging into the body cavity. Three sectioned nerves are apparent in the uninvolved portion of the kidney, just above the nodule; × 1.

time with the progressive obliteration of blood vessels. The living tissue of the lesion is distended with parasites so that it virtually constitutes a reticulum. The blood vessels are dilated. The striated muscle fibers at the periphery of the lesion are swollen, stain less intensely, appear fragmented and are evidently disintegrating.

The reaction to the parasites consists chiefly of their phagocytosis by the endothelial cells which eventually form giant cells. The latter react not only to the parasites, but also to the necrotic tissue, around which they form a layer sequestrating it from the living tissue. In addition to the endothelial cell reaction, there is a rather marked infiltration of the necrotic tissue with polymorphonuclear leukocytes, but these cells are rare elsewhere except in the vicinity of blood vessels. Eosinophils and lymphoid cells occur in variable numbers at the border of the lesion. The reaction of the tissues evidently checks the multiplication of the parasite. Certain lesions studied consist to a large extent of massed giant cells in which nearly all the parasites are included. In some cases the protective reaction has gone so far as to reduce the lesion to a sequestrum of necrotic material surrounded by actively forming granulation tissue. It is a notable fact that considerable numbers of well preserved parasites persist in the spaces of the fibrinoid reticulum after they have disappeared from the living tissue of the lesion.

The pulmonary lesions vary in appearance in accordance with their size. The larger lesions, some of which measure 2 cm. or more in diameter, are composed of centrally situated, firm, dry, dull yellowish or pinkish material, and an external layer of soft, gelatinous, grayish tissue, with a more or less reddened periphery (see fig. 3). Smaller lesions are of soft consistence, of grayish color with reddening of the surrounding lung tissue and show little or no necrosis. The macroscopic characteristics of the larger lesions are thus quite similar to those of the subcutaneous lesion. The degree of lung involvement varied in different cases, but was extensive in all inoculated turkeys examined. The amount of lung tissue capable of functioning appears in some instances to have been reduced to a minimum. In extensively involved lungs, the lesions often become confluent, sometimes forming in the larger turkeys caseous masses 4.5 cm. in diameter. In a turkey weighing 2,020 gm., one of the lungs which was extensively involved weighed 45 gm. The process is found at times extending from the larger lesions to the thoracic wall with production of lines of opaque

induration in the intercostal muscles, extending parallel with the ribs. Attention was at first directed to the study of the subcutaneous lesions of the inoculated turkeys, and the lungs were not examined with special care in the first two turkeys to succumb. Each of the other 12 showed extensive lung involvement.

Microscopic examination of the lungs showed a process similar in many respects to that found in the subcutaneous lesion but modified by anatomic structure. The older portions of the lesions consisted of necrotic lung tissue and more or less fibrinoid reticulum enmeshed in which were numerous polymorphonuclear leukocytes and variable numbers of the parasite. Necrotic blood vessels were greatly distended with blood. As the parasites migrate from the early foci, they first infiltrate the peripheral zone of the lung lobule, and to a less extent the portions adjacent to the infundibular space so that a median zone of the lobule is for a time uninvolved. Usually most of the parasites that occur in the infundibular or bronchial spaces are included within cells, but in one case in which there was a rapidly developing dyspnea, the larger air spaces as well as the tissue showed great numbers of the blue staining, invasive forms. The cellular reaction in the involved lung is similar to that seen in the subcutaneous lesion, but there is, in addition, a variable amount of serous and fibrinous exudate into the air spaces. In fact, extensive pneumonia may frequently occur in portions not infiltrated by the parasite. The pneumonic lung may show edema and infiltration of the alveolar walls associated with atelectasis. The pulmonary lesions resemble the primary lesion in respect to the giant cell reaction to the parasite and necrotic tissue and to the peripheral cellular infiltration. Pneumonia may cause death in cases in which the parasite has been for the most part destroyed, so that it appears that the involvement of the lung is on the whole more fatal than that of the liver.

Lesions of the liver were found in 8 of the 14 turkeys inoculated, which is relatively infrequent as compared with their occurrence in the natural disease. In one of these turkeys only a single liver lesion was found, in none were there more than 6 present, and in the entire series a total of only 28 lesions was noted. These nodules were usually much smaller than the larger of the lung foci, but in two instances they were of equal size. The small size of the majority of the liver lesions indicates that they are for the most part secondary to the larger lung lesions; the occasional larger ones probably result from the simultaneous invasion of the liver and lung. It is quite evident

that the liver lesions are of the same general character in both the natural and the inoculated disease, so that further description of them in the latter is unnecessary. The distribution of the lesions in this infection is evidently determined by the factors governing the dissemination of the parasites, which will be discussed later.

Three of the inoculated turkeys developed lesions of the kidney. In one there was a large tumor-like nodule measuring 4x3.3x2.5 cm. in diameter (see figs. 4 and 5). This nodule had a smooth, rounded surface, and on incision appeared to be composed of soft, dull pinkish tissue, without markings. In its gross appearance the substance of this nodule simulated closely lymphoid tissue or that of a lymphoma. The kidney lesions in the other two cases were considerably smaller and appeared in the surface of the kidney as grayish, slightly elevated areas. From the diffuse character of the lesions and the abundance of organisms present, it is apparent that the kidney furnishes a favorable medium for the development of the parasite of blackhead.

The kidney lesions all show microscopically an active acute process. Although they may attain a much greater size than the lesions of the lungs and liver (see fig. 4), they differ from these and from the subcutaneous lesion in that there are only microscopic foci of necrosis, and fibrin is present in small amounts or absent. In one case the kidney appeared normal at postmortem examination, but showed a microscopic lesion in stained section. Early lesions show infiltration with great numbers of the pale, blue staining invasive forms of Histomonas, with a consequent crowding apart and destruction of tubules. Loss of parenchyma is associated with edema of the connective tissue. The cellular reaction is here as elsewhere, chiefly endothelial in type, and giant cells which are present in the early lesions, later occur in great numbers. Numerous endothelial cells are found in the process of mitotic division. Eosinophils occur in variable numbers chiefly around degenerating tubules. Leukocytes of other types are few in number. The parasites may occasionally be found within tubules, both free and within giant cells. They are distributed singly or in closely packed masses throughout the tissue, but do not occur in nests as in the cecal lesions.

The brain and spinal cord of one of the inoculated turkeys which developed a peculiar gait, with legs straightened and stilt-like, body carried horizontally, and wings held so that the back appeared flattened — were examined, but there was no macroscopic lesion to account for the peculiar symptoms.

Symptoms.—The period of incubation is found to be remarkably constant notwithstanding great discrepancy as to age and size of the various turkeys at the time of inoculation. Several of the 7 turkeys that were inoculated at the age of 48 days — when they weighed from 350 to 510 gm. — showed a slightly longer incubation period than the last one which was inoculated when 104 days old, and weighed 2,670 gm. Seven of the 14 turkeys inoculated showed the first symptoms 11 days later, two 12, two 14, two 15, and one 17 days later. It is evident that symptoms make their appearance only after vital organs have become extensively diseased. The first sign to be noted is a tendency to lag behind the rest of the flock, evidently a symptom of weakness. This is soon followed by more pronounced weakness, as shown by the slow careful gait with drooping wings, and the tendency to stand in one spot with the head under a wing. Other symptoms may appear at once or later, and without any regular sequence. Loss of appetite frequently appears almost simultaneously with the earliest sign of weakness. In some cases the appetite is capricious and varies from time to time — one turkey showed an abnormally voracious appetite. Loss of weight is practically concomitant with loss of appetite, and appears to occur invariably in the inoculated as well as in the natural disease. The effects of the inoculated disease on the growth of the turkeys employed, together with other data are illustrated in the appended chart.

During the summer of 1919, as well as in 1918, no loss of weight was recorded except in cases of blackhead. Sulphur colored droppings do not appear as regularly in the inoculated as in the natural form of the disease, but were noted in several of the inoculated turkeys. Since they have appeared in cases of inoculated blackhead, in none of which was there involvement of the cecum, it is obvious that they result from the derangement of the function of some other organ. It is probable that this change in the character of the excrement is in some way dependent on the involvement of the liver, but additional data are necessary to definitely establish this point.

Pulmonary symptoms appear rather late in the course of the disease produced by inoculation and amount at first to a slight cough which is increased by unusual exertion. A pronounced dyspnea develops in some cases late in the disease. The turkey then squats with beak and throat widely distended, making it possible to view the interior of the trachea through the distended larynx. Pronounced

dyspnea was not noted in many of the series, although the majority showed extensive involvement of the lung. Paleness of the skin about the head may become marked as the disease progresses, and in several was noted with the earliest sign of weakness. The earliest death resulting from the subcutaneous inoculation of blackhead virus thus

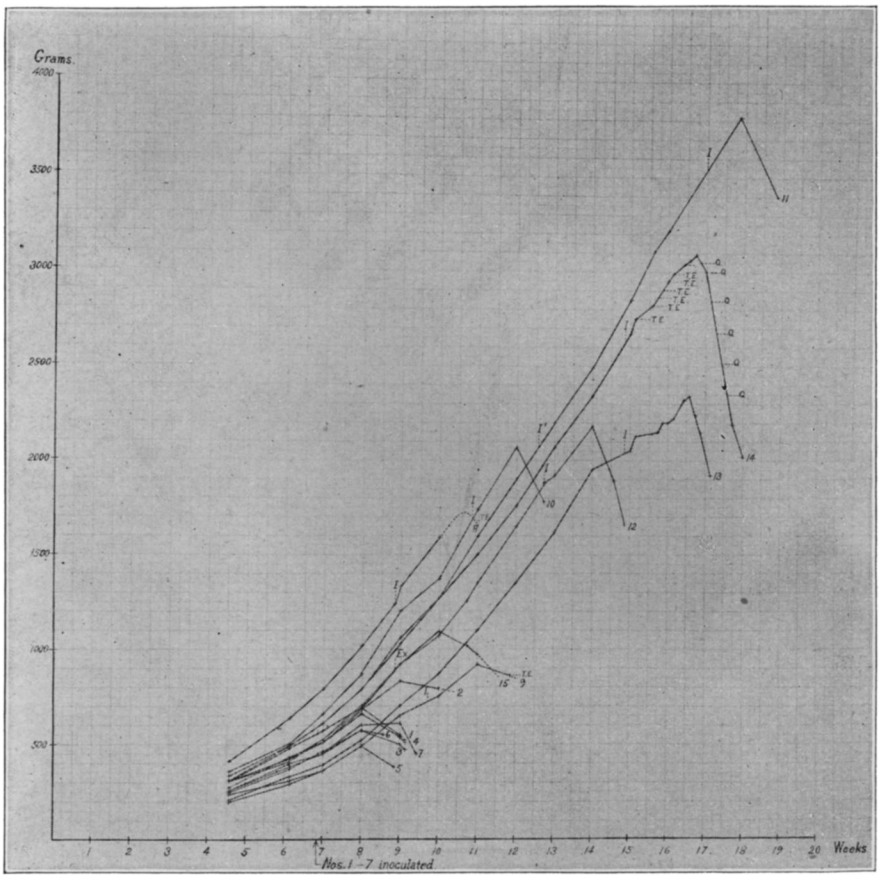

Chart 1.—Numbers at ends of curves are numbers by which turkeys are designated. I., inoculation with blackhead virus; I.'. inoculation with sequestrum from chicken; Ex., exposure to respiratory discharges of inoculated turkeys. T. E., injection of tartar emetic; Q., injection of quinin dihydrochlorid.

far observed, occurred on the twelfth day, the latest on the twenty-fifth, the average being a little less than seventeen days.

The more important data of the various experiments on the inoculation and exposure of turkeys are given in table 1.

TABLE 1.—RESULTS OF INOCULATION OF TURKEYS

Date, Source of Virus	Turkey*	Age and Weight	Inoculation	Symptoms	Result	Loss from Maximum Weight, Gm.	Remarks
Inoculation 1	1	48 days 550 gm.	Subcutaneous	Marked weakness after 11th day	Died 14th day	170	Large local subcutaneous lesion. Secondary lesions in lungs and liver. Microscopically process older in lung than in liver
	2	48 days 520 gm.	Subcutaneous	Weakness about 17th day; cough 22d day	Dying. Killed on 25th day	40	Involvement of lungs extensive; liver shows 3 lesions and kidney one large lesion
Inoculated Aug. 14, 1919, with liver lesions of spontaneously infected turkey (C. P. S. 19.48	3	48 days 460 gm.	Subcutaneous	No note	Died 16th day	100	Involvement of lungs extensive; liver, 2 minute lesions
	4	48 days 510 gm.	Subcutaneous	Developed peculiar gait on 11th day	Killed 16th day	150	Involvement of lungs extensive; one lesion against vertebral column. No nerve lesions found
	5	48 days 365 gm.	Subcutaneous	Extreme weakness on 14th day	Killed 14th day	110	Liver and ceca normal. Lungs not examined
	6	48 days 450 gm.	Subcutaneous	Dumpish on 11th day	Died 12th day	20	Liver and ceca normal. Lungs not examined
	7	48 days 450 gm.	Subcutaneous	Pale and dyspneic on 15th day; lively	Died 18th day	160	Involvement of lungs extensive; liver, 4 small lesions
Aug. 25, turkeys 1, 2, 3, 4, 5, 7 (coughing Aug. 27)	15	63 days 920 gm.	Exposed to sick inoculated turkeys	Weakness and sulphur colored droppings on 15th day	Died 22d day	120	Lesions in one of the ceca and in liver. Kidneys extensively involved (microscopically). Lung, minute microscopic lesion with organisms
Inoculation 2 Aug. 28 from turkey 5	8	62 days 1,240 gm.	Subcutaneous	Wings droop, sulphur colored droppings on 11th day	Died 15th day	No note	Death followed injection of tartar emetic. Involvement of lungs extensive. Liver, 4 small lesions
Sept. 3, from turkey 6570 (spontaneous case)	9	68 days 750 gm.	Subcutaneous	Weakness and coughing few days before death	Killed 15th day	60	Killed after injection of tartar emetic. Involvement of lungs extensive, other organs normal. Microscopically excessive reaction. Few remaining organisms isolated
Inoculation 3 Sept. 11, from turkey 8	10	70 days 1,740 gm.	Subcutaneous	Weakness on 11th day	Killed 13th day	290	Marked dyspnea. Involvement of lungs extensive; active process (microse). Liver, 2 small lesions
Inoculation 4 Sept. 24, from turkey 10	12	89 days 1,880 gm.	Subcutaneous	Pale and weak on 11th day; refused food	Died 15th day	520	Involvement of lungs. Liver normal. Kidney one early lesion with organisms in large numbers
Inoculation 5 Oct. 9, from turkey 12	13	104 days 2,050 gm.	Subcutaneous	Weakness and yellow droppings on 13th day	Killed 16th day	420	Involvement of lungs extensive; diffuse pneumonia (microscopic chronic picture). Liver, several lesions; kidney, 2 lesions (extensive acute process microscopically)
	14	104 days 2,670 gm.	Subcutaneous	Pale, quiet on 14th day	Killed 23d day	1,070	Received injection of tartar emetic and quinin (see text). Large local lesion. Involvement of lungs extensive; necrosis of breast muscle at sites of inoculation
Sept. 23, from sequestrum from an inoculated chicken	11	88 days 2,200 gm.	Subcutaneous (chicken material)	None	No evident disease		Subcutaneous sequestrum persisted for weeks
Inoculation 6 Oct. 25, from turkey 13	11	120 days 3,590 gm.	Subcutaneous	Weak on 11th day; refused food	Killed 13th day	420	Local lesion small; involvement of lungs extensive; nodules and pneumonic areas (microscopically); liver, 1 lesion (1 cm.)
Oct. 9 and 25 given flies fed on lesions	16	1½ mos. 740 gm.	Fed on "blow" flies	None	No evidence of disease		Made normal gain in weight
Nov. 9. Exposed to hens	16	2½ mos. 1,650 gm.	Exposed to hens	Weakness and yellow droppings on 22d day	Killed 24th day	330	Extensive involvement of right cecum. Liver, numerous depressed lesions (microscopically showing early infection)

* All turkeys in this column were 19: 19-1, 19-2, etc.

The inoculation of other species of birds and of laboratory animals with the virus of blackhead serves to demonstrate their relative non-susceptibility. Since blackhead occasionally occurs spontaneously in the common fowl,[2, 4] it might be expected that the subcutaneous inoculation of the virus would be attended with positive results in this species.

Chicken.—A series of 2-months-old White Plymouth Rock chickens, inoculated subcutaneously, intramuscularly, intravenously and intraperitoneally, respectively, failed to develop the disease. Several white Leghorns inoculated subcutaneously at the ages of 3½ to 4½ months, showed no definite local reaction and remained normal. The subcutaneous and intracecal inoculation of a number of Rhode Island Red chickens, some 2 and some 3½ months old, produced no evidence of disease. In such of the chickens as were killed or operated on, a flake of dry yellowish material was found at the site of inoculation. Such material, recovered 25 days from the time of inoculation of chicken (19-VI), and implanted in a turkey (19-11), failed to produce disease, indicating the death of the virus.

Four chickens, 48 hours after hatching, were inoculated with tissue of blackhead lesions. One of these chicks (19-4), showed a reaction at the site of inoculation 6 days later, and on the next day the lesion, appearing as a well defined, flat topped mass, was excised. It measured about 1 cm. across and 4 mm. in thickness, and was found to be composed of tough yellowish material surrounded by a ring of soft almost gelatinous pinkish tissue. (The inoculation of this material into 4 chicks of the same lot now 9 days old, produced in some, definite but transient reactions. See table 2.) The operation wound healed promptly, and there was no evidence of local recurrence of the disease. Sixteen days after the inoculation, the breathing was somewhat labored. The chick was killed, and pale grayish, rather indefinitely outlined lesions were found in both lungs. Histomonas meleagridis was demonstrated in fresh preparations from these foci. Of the other 3 chicks inoculated when 48 hours old, 1 showed a definite reaction after 7 days, and the other 2 after 9 days. Necrosis of the central portion of the lesions was apparent from the first, and this rapidly increased, forming a crust which involved the overlying skin. In one (chick 19-3) the process continued to extend up to the fourteenth day and formed a nodule of considerable size, but in the other two, regres-

[4] Milks, H. J.: Louisiana Agric. Exper. Station Bull., 1908, 108, p. 1.

sion followed soon after the development of the mass. In none of these was there evidence of involvement of internal organs. The transfer of the virus by the implantation of portions of the excised subcutaneous lesion of chick 19-4 into four 9-day-old chicks resulted in less well defined lesions. The subsequent inoculation of lung lesions obtained from chick 19-4 into 2 chicks 18 days old, resulted negatively.

Stained sections of the 6-day excised lesion of chick 19-4 showed acute inflammation with polymorphonuclear leukocytes in large numbers, but no necrosis. Moderate numbers of the parasite are present. The lung shows large foci of necrosis with extensive cellular infiltration, chiefly endothelial in type, and a few parasites. In general, the tissue reaction of the chicken to the virus seems to be of a more acute exudative character than in the turkey.

From the inoculation of chickens, it would appear that the virus develops for a time in very young chicks, but is soon destroyed by the reaction of the tissues. Involvement of the lungs occurred only in one from which the subcutaneous lesion had been excised. Even in slightly older chickens the virus fails to produce well defined active lesions. It was impossible to demonstrate the presence of virus in a 4 months chicken, 25 days after its inoculation, by the transference of the implanted material to the susceptible turkey.

Blackhead lesions were fed in large amounts to two 15-day-old chicks. The droppings of both these chicks were free from intestinal parasites and remained so on repeated examination. The microscopic inspection of the cecal contents and scrapings of the mucosa at necropsy, 5 and 8 days later, respectively, failed to show the presence of flagellates or other protozoa.

Table 2 gives the results obtained from the inoculation of very young chickens, but does not include the details of the experiments on older chickens, which invariably resulted negatively.

Pigeon.—The subcutaneous and intramuscular inoculation of pigeons has resulted in the production of a well marked but transient process in a certain proportion of cases, and slight or questionable reactions in several others. The virus was twice carried in pigeons to the second transfer, and once to the third transfer from the turkey. The failure to produce lesions on one occasion was evidently due to the employment of too old a lesion for inoculation purposes, for no organisms were distinguished in fresh preparations, and the lesions

had commenced to regress. In all, 29 pigeons were inoculated on 8 occasions. Nine produced well defined lesions, and the parasite of blackhead was demonstrated in fresh preparations from 5 of these. Several others showed slight or ill defined swelling about the implant. Parasites were most numerous in lesions taken on the seventh and eighth days after inoculation. They were found in one lesion on the tenth day, but were not demonstrable in two other lesions commencing to regress, one taken on the ninth and one on the tenth day.

TABLE 2

RESULTS OF INOCULATION OF CHICKENS

Date, Virus	Chicken*	Age	Inoculation	Result	Remarks
Inoculation 1 Oct. 25; from turkey 13	1	48 hours	Subcutaneous	Local lesion; maximum development 10th day	Regression complete by 18th day; no secondary lesions
	2	48 hours	Subcutaneous	Local lesion; maximum development 11th day	Regression advanced by 14th day; no secondary lesions
	3	48 hours	Subcutaneuos	Local lesion: maximum development 14th day	Transformed to a crust; removed on 23d day, nearly healed
	4	48 hours	Subcutaneous	Local lesion; excised 7th day; active; numerous parasites	Wound healed rapidly: on 16th day labored breathing, killed; lesions in both lungs; parasites present
Inoculation 2 Nov. 1; from chicken 4	5	9 days	Subcutaneous	Small local lesion	Regression noted on 12th day
	6	9 days	Subcutaneous	No reaction	
	7	9 days	Subcutaneous	Well defined local lesion	Regression by the 12th day
	8	9 days	Subcutaneous	No definite reaction	Minute mass
Nov. 7 and 8; fed with lesions of turkey 11 and pigeon 18	9	15 days	Fed lesions	Negative	No flagellates appeared in feces
	10	15 days	Fed lesions	Negative	No flagellates appeared in feces
Inoculation 3 Nov. 10; from lung lesion, chicken 4	11	18 days	Subcutaneous	Small local lesion	Early regression
	12	18 days	Subcutaneous	Negative	

* All chickens in this column were 19: 19-1, 19-2, etc.

The local lesion develops at about the same rate in pigeons as in turkeys, but quickly regresses. Definite swelling, which appears around the implant 5 or 6 days after inoculation, increases rapidly for 2 to 4 days, and then quickly subsides. The process is thus at its height in 7 or 8 days, and is regressing 10 days after inoculation. The early 1-day lesion is found on section to consist of a small central sequestrum, representing the implanted tissue, surrounded by grayish opalescent tissue of soft consistence, evidently degenerated breast

muscle. More advanced lesions taken from 7 to 9 days after inoculation consist largely of firm, dry necrotic tissue, of dull pinkish color, more or less enclosed in a layer of opalescent grayish tissue which is not definitely demarcated from the surrounding muscle. In superficially situated lesions, the necrotic portion is apparent through the skin as a depressed central area, and this is surrounded by a ring of living tissue making the border of the lesion rounded and elevated. Regression soon follows and is attended with the subsidence of infiltration and the formation of sequestrum which becomes slowly absorbed. In no case did the disease spread to other parts of the body.

Microscopically, the inflammatory reaction is of a distinctly acute type and is even more pronounced than that observed in the newly hatched chick. In contrast with the reaction of the tissues of the turkey, purulent exudation is a prominent feature of the process. The parasites in the active 7-day lesion occur in a zone around the centrally situated necrotic tissue, and this in turn is surrounded by a thick layer of cellular infiltration. Peripheral to this between the separated muscle fibers are newly formed connective tissue and blood vessels. By the tenth day after inoculation, the parasites may have wholly disappeared and the lesion presents a sequestrum enclosed in a layer of lymphoid and endothelial cells.

It is thus possible to produce in pigeons a transient but well defined localized blackhead infection. About 30% of those inoculated developed active lesions and, if the cases are eliminated which were probably inoculated with nonvirulent material, the positive cases are raised to 39%. It is quite possible that blackhead might be transmitted in pigeons indefinitely by inoculation if active 7 or 8 day lesions were used for implantation, and a sufficiently large series of this species were employed. The abstracts of the experimental inoculations of pigeons are given in table 3.

Mammals.—Several species of mammals were also inoculated with the same material used in inoculating turkeys, chickens and pigeons. Rabbits were inoculated subcutaneously on the side and on the ear, intraperitoneally, and also in the testicle without success. A minute abscess which developed at the point of inoculation beneath the skin of the ear was excised 20 days after inoculation and sectioned, but no organisims were found. New-born guinea-pigs were inoculated subcutaneously and intraperitoneally with negative result. A small swelling, appearing in one case at the site of subcutaneous inoculation, was

excised and found to consist of necrotic material with no demonstrable parasites. Common mice and young Japanese waltzing mice were inoculated subcutaneously. Although small swellings appeared beneath the skin of the latter animals, stained sections showed only an acute inflammatory reaction to the foreign tissue and no parasites. Mammals have thus proved invariably nonsusceptible to the virus of blackhead.

TABLE 3

RESULTS OF INOCULATION OF PIGEONS

Date, Virus	Pigeon*	Inoculation	Killed	Result	Remarks
Inoculation 1 Oct. 9, 1919, from turkey 12	1	Subcutaneous	5th day	+	Slight local infiltration
	2	Subcutaneous	8th day	++	Large local lesion; numerous parasites
	3	Subcutaneous	.....	+	Transient reaction
	4	Subcutaneous	16th day	0	
Inoculation 2 Oct. 17, from pigeon 2	5	Intramuscular	.....	0	Slight local reaction
	6	Intramuscular	10th day	++	Indurated mass regression; no parasites
	7	Intramuscular	.....	0	
	8	Intramuscular	.....	0	
Inoculation 1a Oct. 25, from turkey 13	9	Intramuscular	7th day	++	Microscopically active lesion, many parasites
	10	Intramuscular	.....	0	Indefinite swelling
	11	Intramuscular	9th day	0	
	12	Intramuscular	.....	0	Indefinite swelling
Inoculation 3 Oct. 27, from pigeon 6	13	Intramuscular	.....	0	Virus probably not present in material inoculated
	14	Intramuscular	.....	0	
	15	Intramuscular	.....	0	
	16	Intramuscular	.....	0	
Inoculation 2a Nov. 1, from pigeon 9	17	Intramuscular	14th day	0	No definite local reaction
	18	Intramuscular	7th day	++	Large active local lesion; parasites present
	19	Intramuscular	14th day	0	Indefinite swelling on 11th day
	20	Intramuscular	14th day	0	No local reaction
Nov. 7, from lung lesion turkey 11	21	Subcutaneous	.....	+	Definite swelling from 5th to 8th day
	22	Subcutaneous	8th day	++	Large lesion; parasites numerous
	23	Subcutaneous	10th day	++	Regressing
Inoculation 3a Nov. 8, from pigeon 18	24	Subcutaneous	.....	++	
	25	.....	9th day	++	Regressing
	26	.....	.....	++	
	17	.....	.....	0	
Nov. 10, from chicken 4	28	Intramuscular	.....	0	
	29	.....	.....	0	

* All pigeons in this column were 19: 19-1, 19-2, etc.

THE INFECTIOUS CHARACTER OF INOCULATED BLACKHEAD

Prior to the discovery of lung lesions in the inoculated turkeys, it was assumed that the virus was confined to the nonulcerated subcutaneous lesions and was not discharged from the body. The 7 turkeys first inoculated were accordingly kept with the 8 normal ones. As

soon as it was determined that the lungs were involved, the question arose as to the possibility of the virus being discharged in mucus from the respiratory tract. That the disease may be transmitted spontaneously from inoculated to healthy turkeys is shown by the following data:

Of the brood of 15 young turkeys hatched on June 27, 1919, 8 were inoculated subcutaneously on Aug. 14, but were not isolated from the others of the flock. On Aug. 25, several of the inoculated birds were already showing weakness and loss of appetite, and within a few days there was more or less coughing among the inoculated birds, which continued until death. There was thus a possibility of contamination of the food and drink with the discharges from the respiratory tracts of the inoculated turkeys from Aug. 27 on. On Sept. 9, twelve days later, one of the uninoculated turkeys (19-15) passed sulphur colored droppings. The weight taken on the following day showed a loss of 70 gm. in the preceding 5 days. Death occurred on Sept. 16. The wall of one of the ceca presented several markedly thickened areas, ulcerated but without sequestrum or notable necrotic tissue. This cecum contained thick fluid of a reddish chocolate color. The other cecum appeared normal. The liver presented numerous characteristic lesions, many of which measured about 1 cm. in diameter. In the kidneys were several ill-defined, pale areas.

Stained sections of the diseased cecum showed extensive infiltration of the tissues with Histomonas meleagridis. There were no demonstrable flagellates in the cecal glands or on the surface of the mucosa. There were large numbers of Blastocystes in the cecal contents. Sections of the liver show characteristic lesions of blackhead. The kidney was extensively infiltrated with parasites. The involved areas were not well circumscribed. Some of these measure 5-8 mm. in diameter and appeared to be derived from the coalescence of smaller lesions from 1-2 mm. in diameter. Large numbers of the organisms were present, in association with extensive cellular infiltration but there was no necrosis. In the lung a minute microscopic lesion containing blackhead parasites was found.

Since this is the only case of spontaneous blackhead which occurred in the flock during the season, and since the first symptoms were noted 12 days after the appearance of coughing in the inoculated turkeys — an interval which coincides to a day with the average period of incubation in the inoculated disease and, as far as it has been ascertained, with the average incubation period of spontaneous blackhead — it appears most probable that this turkey became infected with the discharges from the respiratory tracts of the inoculated turkeys. That the contagion in this instance was derived from the lesions of the liver or kidney of the inoculated birds seems extremely unlikely, for not only were these lesions few in number, but organisms would probably be discharged from them much less readily than from

the infected lung. Furthermore, the probability of contamination of food and drink by respiratory discharges is much greater than by fecal material.

ATTEMPTED TRANSMISSION BY FLIES

Since young turkeys feed regularly on "blow flies" which collect on the droppings and especially on the cecal discharges, it was thought that these insects might act as distributors of the virus of blackhead. Flies of the species Calliphora erythrocephala Wied, were reared in captivity from eggs deposited on exposed strips of beef. The flies after emerging from the pupal cases were fed on sweetened water until used in this experiment.

On Sept. 15, a young turkey (19-16), about a month old but distinctly undersized, was obtained from a source where there had been more or less blackhead, although not in the brood from which this one was taken. This turkey was kept under observation in a brooder provided with a small wire enclosure wholly isolated from other turkeys and common fowls. Once or twice each day it was taken to an adjoining field to feed on grasshoppers and crickets. This turkey grew normally and showed no evidence of disease.

On Oct. 9, about 70 flies were fed with finely minced lung lesions and a portion of the subcutaneous lesion of an inoculated turkey, which were greedily devoured. Five hours later these flies were transferred to a clean cage in which they were transported about 15 miles, and were fed to the turkey 2 hours later. Sixty-eight of the flies were devoured and no other food except a few blades of grass were furnished until the next morning. There was no evidence of infection following this procedure which was again repeated on Oct. 25 when 67 "blackhead-fed" flies were devoured. The turkey remained normal and continued to grow rapidly for the next 15 days, indicating that the ingestion of the flies was evidently without effect.

EXPOSURE TO COMMON FOWLS

On Nov. 9 the turkey, which had been previously fed with infected flies, was placed with a small flock of hens on the same premises on which turkeys had been exposed to hens a year before. Twenty-two days later— on Dec. 1 — the first symptoms of blackhead appeared, and characteristic lesions of the ceca and liver were found on killing the bird on Dec. 3. Either the virus was present in this turkey for 37 days from the last feeding of flies without producing symptoms, or the disease was derived in the shorter period of exposure to hens. The latter explanation appears to be the more plausible, since the incubation period, in so far as it has been determined, appears to vary within comparatively narrow limits, i. e., between 11 and 17 days.

TREATMENT OF THE INOCULATED TURKEY WITH TARTAR EMETIC AND QUININ

In the summer of 1918 an unsuccessful attempt was made to prevent infection with blackhead by the administration of chaparro amargosa, a drug which has proved efficient in the treatment of amebic dysentery in man.[5] During the past season further experimentation along this line was undertaken, employing certain drugs known to be of value in the treatment of certain protozoan infections of the human being.

On Oct. 9, 1919, turkey 19-14, at the age of 104 days, was inoculated subcutaneously on the left breast with a bit of the subcutaneous lesion of another turkey (19-12), and on the right breast with a bit of similar material which had been kept frozen for 5 minutes previous to its implantation. The body weight on the following day was 2,670 gm.

On Oct. 11, 1 c c of a 1% aqueous solution of tartar emetic was injected into the wing vein. This was followed immediately by pronounced symptoms, i. e., panting, drooping of wings and unsteady gait—the latter persisting for about one hour.

The same dose of tartar emetic was injected on Oct. 14, 15 and 16, and 1.5 c c on Oct. 17, 18 and 20, more or less pronounced symptoms following each injection. In all, 85 mg. of this drug were injected. Swelling appeared around the implant on the left breast 5 days after its inoculation and a well defined lesion appeared which increased rapidly in size during the treatment with tartar emetic. Trichomonads, which had been noted in the cecal discharges previous to treatment, persisted in undiminished numbers. The body weight showed a steady increase, being 3,090 gm. on Oct. 22.

The first symptom of blackhead, i. e., failure to eat, appeared on Oct. 23, fourteen days after inoculation. The weight showed a drop to 3,050 gm. On this date, 1.5 cm. of a 10% solution of quinin hydrochlorid was injected, in part into the wing vein, in part into the breast muscle. On the following day the turkey developed a slight cough. On Oct. 24, 25, 26, 27 and 28, 1.5 cm. of the aqueous solution was injected, after which all treatment was discontinued. The total amount of quinin injected was about 900 mgm. The cough increased in frequency, and the disease showed steady progress during the treatment with quinin. After being carefully nursed for several days, the turkey appeared to be dying and was killed on Nov. 1, 23 days after inoculation.

Postmortem Examination.—Weight, 2,020 gm. (loss, 1,070 gm. from maximum weight attained); the primary lesion on the left breast measured 8 x 4 x 1.5 cm., and showed proportionately less necrotic material and a larger amount of pinkish translucent tissue than is usually seen in such lesions. Histomonas meleagridis was found in large numbers in fresh preparations. Posterior to the primary lesion was a pocket filled with chocolate colored fluid. The breast muscle at points where the injections were made showed in some instances glistening whitish areas, frequently several centimeters across. The lungs, with the exception of the apices, were for the most part replaced by large confluent lesions. One such lesion measured 4.5 x 4.5 x 3 cm. The left

[5] Tyzzer. E. E.: Jour. Med. Res., 1920, 41, p. 211.

lung weighed 45 gm. and the right 34 gm. In the mesentery were opaque masses of the size of a small pea, and on the surface of the left kidney were minute opaque grains about 0.5 c c in diameter. The liver, ceca and kidneys showed no evidence of involvement.

Microscopically the subcutaneous lesion showed a much thicker layer of reaction tissue infiltrated with organisms than had been observed in any of the other inoculated turkeys. The organisms were not only present in great numbers, but showed less evidence of degeneration than is usually encountered in the disease. The small lesions in the mesentery and on the surface of the kidney were not characteristic of blackhead, and may have resulted from the injection of one or the other of the drugs employed.

It is apparent from these results that neither tartar emetic nor quinin is of any therapeutic value in blackhead. The primary lesion showed, if anything, greater development than usual, and the course of the disease was not appreciably modified. The implantation of a bit of lesion which had been frozen for five minutes failed to produce an active lesion.

DISCUSSION

The experiments here outlined demonstrate the uniform susceptibility of turkeys to blackhead. The regularity of the inoculated disease irrespective of weather conditions or the size, and as far as has been determined, the age of the turkey, indicates that all that is necessary for the production of the disease is the entrance of the parasite into the tissues. Whether conditions which are supposed to lower the resistance of these birds, or functional disturbances of any kind, which are possibly more frequent in early life, tend to favor the entrance of the parasite is not known. It appears possible, from what has been learned of the nonresistant properties of the latter outside of the body and of its behavior when introduced into the tissue, that those conditions which are supposed to lower the resistance of the turkey in reality are less destructive to the parasite and so favor its transmission.

Of the other species employed in these experiments, none approach the turkey with respect to susceptibility. Since ruffed grouse reared in captivity commonly succumb to this disease, it is quite probable that this species may be as susceptible as the turkey. The common quail is also somewhat susceptible. It is rather remarkable that the common fowl should prove so refractory to the infection, especially as spontaneous cases have been occasionally observed in this species. It is difficult to account for such cases — possibly certain breeds or stocks are more susceptible; their resistance may be lowered by unfavorable conditions; or more virulent or adapted strains of the blackhead para-

site may be present. The disease has been produced by the inoculation of young chickens and, although in most cases it was self-limited, in one case it involved the lungs. A transient local infection in pigeons may be produced by inoculation, but the mammals inoculated have proved invariably nonsusceptible.

The virus employed has remained remarkably constant, the incubation period has not altered, and there has been no diminution of virulence in the course of these experiments.

The conclusion reached by Hadley [6] that blackhead is caused by the invasion of the tissues with an intestinal flagellate, Trichomonas, species undetermined, lacks satisfactory proof. From his descriptions and illustrations it is apparent that he had under consideration a flagellate of the genus Trichomonas (figs. 1 and 2), an organism of the Blastocystis type (figs. 11, 12 and 29), and the tissue parasite which had been previously described by Smith as the causal agent of blackhead. These are regarded, on the basis of transitional forms and of topographical relationship to goblet cells or breaks in the epithelium, as developmental stages of a single species. The flagellated forms are said to measure from 8-12 microns in length, and to possess three anteriorly, and one posteriorly, extending flagella, an undulating membrane, axostyle, chromatic line, chromatin blocks and other features, all of which correspond rather closely with the description of Trichomonas eberthi Martin and Robertson. Multiplicative processes are described by this author, however, which are without analogy in other species of Trichomonas, so that it does not appear unreasonable to question whether he has not mistaken several mingled species for developmental forms of a single species.

In the present study of a large series of cases of blackhead no morphologic evidence has been found suggesting the transformation of the associated cecal trichomonad into the organism of blackhead. Although the latter parasite is found contiguous to, and occasionally on, free surfaces, forms of it have never been observed which would suggest its identity with Trichomonas eberthi which occurs in the cecal glands. In the study of stained sections of the infected lungs, an occasional example of Histomonas meleagridis was observed in which the blepharoplast and radiating filaments were situated at the surface of the cytoplasm at a distance from the nucleus, but such forms were not only morphologically unlike any of the flagellates thus far found

[6] Agric. Exper. Station, Rhode Island State College Bulls. 166 and 168, 1916.

in the cecal glands, but were also much larger. Although the movements of the blackhead parasite as observed in the warm chamber as well as its morphology, indicate a close relationship to the trichomonads, no evidence of its identity with any of the established species of the latter is at present available. The study of examples of the parasite which have passed from the tissues into the air spaces of the lung, the bile ducts, and the urinary tubules, has thus far revealed none which has assumed the morphology of the flagellated trichomonad. It has not yet been demonstrated that this parasite can multiply in the lumen of the intestine or elsewhere outside the tissues. Young chickens free from intestinal protozoa were fed large amounts of blackhead virus, with a view to obtaining a flagellated stage of the parasite in the intestine. Entirely negative results followed, no flagellates or other protozoa being demonstrable during life or on post-mortem examination of the ceca.

Although Hadley claims that there are transitional stages between the flagellated Trichomonas of the cecal glands and the tissue parasites, he has furnished no satisfactory evidence of such transformation. The presence of numerous flagellates beneath the epithelium, without associated tissue reaction, may only be accounted for by post-mortem invasion or by artefact in the process of preservation. Their occasional occurrence in diseased or necrotic tissue may be accounted for by secondary invasion. Under these circumstances they are promptly taken up by phagocytes, but instead of assuming the appearance of Histomonas, retain their characteristic trichomonad morphology. The presence of Blastocystis beneath the surface of the cecal mucosa [7] indicates that cecal contents have in some way been forced into the tissue beneath the dislodged epithelium.

Invasion of the tissue is said by him to be preceded by excessive multiplication of the flagellates and "the swarming of the motile stages from the cecal content into the crypts, in such numbers as to menace the integrity of the epithelial wall." The absence of flagellated organisms from the ceca in certain acute cases of blackhead does not conform with this view. In turkeys 19-15 and 19-16, of the present series, no flagellate was demonstrable in either the cecal contents or the glands. Furthermore, the appearance of great numbers of trichomonads in the cecal discharges of turkeys reared at the Medical School in the summer of 1918, showed no significant time relationship to the occurrence of blackhead in this flock. Cases of the disease appeared,

[7] Hadley, P. B.: Agric. Exper. Station, Rhode Island, Bull. 168, Fig. 29.

however, after exposure to infected turkeys and to common fowls, and the time from exposure to the appearance of symptoms is consistent with what is known of the period of incubation in blackhead.

In his consideration of the multiplication of the flagellates in the ceca, Hadley discusses "the typical flagellate dysentery, which is almost invariably the precursor of blackhead." Of the cases of blackhead which have occurred in turkeys reared here under observation during the summers of 1918 and 1919, none has shown diarrhea previous to the onset of the disease. The first symptoms consisted of weakness, loss of appetite, and the appearance of sulphur colored droppings. In young growing birds soft droppings are not abnormal, and the passage of soft, semifluid discharges from the ceca do not indicate dysentery.

By experimental introduction of the parasite into the subcutaneous tissue instead of into the cecal wall, as in spontaneous infection, a disease, inoculated histomoniasis, has been produced, which presents distinct differences from the natural infection. These differences are dependent on the distribution of the lesions — while in spontaneous blackhead the primary lesions are situated in the ceca and the secondary lesions in the liver — in the inoculated disease with the primary lesion beneath the skin, secondary lesions occur constantly in the lungs and occasionally in other organs, and smaller tertiary lesions may occur in the liver and kidney. The distribution of lesions apparently does not depend on the restriction of the growth of the parasite to certain tissues or organs, for as far as it has been ascertained it multiplies readily in any of the soft tissues. Taking the invasive properties of the parasite into account in both the natural and the inoculated disease, the difference in the distribution of lesions is clearly attributable to the relation of the primary lesion to the vascular system.

Thus in the course of the migration of the parasites through the tissues of the cecal wall in spontaneous blackhead, a certain number penetrate the portal veins and are transported to the liver. It is evident that this organ usually serves as an effective filter for the parasites, as lesions have not been reported in the lungs in the spontaneous disease. However, in recently studied cases of spontaneous blackhead, the authors have demonstrated that the organism, after lodging in the liver may subsequently be disseminated to other organs. Early lesions of the kidney with numerous parasites, and in one case a microscopic lesion of the lung containing parasites, have been found in the natural disease.

In the inoculated disease, the parasites multiplying beneath the skin, may penetrate the walls of the systemic veins and thence be carried to the lungs, where they lodge to produce secondary lesions. The lung, however, does not constitute such an effective barrier as the liver to the passage of the parasite, for lesions of the liver and kidney (see fig. 4) are occasionally encountered, which have evidently developed simultaneously with those of the lung. In most cases the lesions of the liver and kidney are of relatively small size as compared with the older lesions of the lung, and may thus be designated as tertiary in character. It appears quite probable that the movements of the lung in alternate expansion and contraction are more favorable to the passage of the parasite through its capillaries than is the case in the liver. The usual failure of the parasite to pass through the sinusoids of the liver makes improbable the occurrence of the hypothetical small forms suggested by Theobald Smith. Its dissemination is evidently quite analagous to that of the cells of certain transplantable, metastasizing tumors of rats and mice, and appears to be wholly through the blood vessels. These facts are consistent with the size and physical characteristics of the known forms of the parasite.

The initial dissemination of the organisms by the blood stream is evidently coincident with the infiltration of the tissue around the subcutaneous implant, which appears from 5 to 7 days after inoculation. It appears reasonable to assume that the size of the lesion is proportionate, at least during the acute phase of the disease, to the number of organisms present. By allowing an equal period of time for the development of lesions in the lung and beneath the skin, dissemination of the parasites by the blood stream would be initiated as early as the middle of the period of incubation, that is, at the time of the appearance of a definite local reaction. The rapid macroscopic changes which take place from 5 to 7 days after inoculation are quite in accord with the multiplication of the parasite by binary division, a point determined by histologic studies.[2] It has already been pointed out by Smith that the initial reaction of the tissues amount to little more than distention by innumerable parasites. Great numbers of the latter evidently perish from the effects of overcrowding during the acute phase of the process; others survive to become phagocytosed by the tissue cells that eventually assume the form of giant cells.

There is no definite evidence of the production of markedly toxic substances by the parasite. Rectal temperatures taken 9 and 15 days

after inoculation showed no definite variation from the normal, as may be seen in table 4.

TABLE 4

RECTAL TEMERATURES 9 AND 15 DAYS AFTER INOCULATION

	Eight Control Turkeys			Seven Inoculated Turkeys		
	Maximum	Minimum	Average	Maximum	Minimum	Average
August 23.....	107.9 F.	106.0 F.	106.69 F,	108.2 F.	105.8 F.	107.2 F.
August 29*.....	107.9 F.	106.5 F.	107.26 F.	107.9 F.	105.3 F.	107.1 F.

* Only five inoculated turkeys survived to this date.

The rapid extension of the subcutaneous lesion is unattended by symptoms, and it is only after vital organs have become seriously diseased that the turkey appears ill or loses weight. It is thus conceivable that spontaneous blackhead might in some instances be arrested without serious impairment of functions, so that cases may occur without symptoms. No evidence of such unrecognized attacks has been obtained in the present investigation, for all the turkeys employed proved to be susceptible to the disease. Neither is there anything to indicate latency of infection, since the incubation period in the entire series showed no great degree of variation.

In this infection death appears to be due to the extensive involvement of vital organs: the lung and less frequently, the liver and kidney in the inoculated disease, and the ceca and liver in the spontaneous disease. Terminal secondary infection evidently occurs for bacteria are frequently obtained in cultures from the liver.

The pathology of the disease, especially with reference to the distribution of the parasites in the body, has a distinct bearing on the question of its transmission. In spontaneous blackhead the virus is largely confined to the ceca, the portal veins and liver. The natural outlet for it, therefore, appears to be the cecal discharges or the bile and thence the feces. In case the kidney is also involved the virus may be discharged in the urine. There is thus slight possibility of its being transmitted by a biting insect, for the virus is rarely present in the circulation outside the portal veins.

In the inoculated disease, on the other hand, the virus passes from the local lesion through certain of the systemic veins, to the lungs, and to a less extent to the liver, and occasionally to the kidney. In this

form of the infection, the chances of its escape through the feces appear slight, as compared with the probability of its discharge with mucus in coughing. The parasite is not discharged in enormous numbers in either form of the disease. They are occasionally found in stained sections within giant cells free on the surface of the cecal mucosa and in the lumina of bile ducts, but have not been identified in the discharges during life. They have been observed on one occasion in mucus from the larynx, but are evidently rare. Stained sections of the lung in the inoculated disease usually show, however, great numbers of cells containing parasites free in the air spaces, but rarely more than an occasional free trophozoite. It is evident that active forms of the parasite seldom leave the tissues, but that quiescent forms may be discharged within the cells which have effected their isolation. It is not definitely determined that the forms, previously interpreted as representing a resistant phase of development, are capable of multiplying when transferred to normal turkeys, although it is quite evident that a large proportion of the organisms are destroyed by the giant cells which appear within the lesions.

Various possibilities may be taken into consideration with respect to the transference of the virus from the disease to normal birds. It is not known at present how long the parasite will remain alive outside of the body. From what has been observed of its characteristics, it appears to be an extremely frail, nonresistant organism, and there is no evidence for the assumption that it lives from one season to another in the soil. Although blackhead was introduced into the yard at the Medical School in the summer of 1918, fifteen turkeys were raised in confinement on the same soil during the following season, without the appearance of any case that could be attributed definitely to this source.

The louse evidently plays no rôle in the transmission of the disease, for not only is Histomonas rarely present in the peripheral circulation, but also the case of spontaneous disease recorded earlier in this paper occurred in an incubator hatched turkey on which there were no lice.

Flies, especially the "blow flies" which feed on the cecal discharges, should be considered as possible agents in the distribution of the parasite, although failure resulted in the single instance in which the transmission through flies was attempted experimentally. These flies feed constantly on the discharges during fair weather and are caught

regularly by young turkeys. If the virus is able to survive even for a short time in or on these insects, their movement from place to place would distribute it.

In a recent paper by Graybill and Theobald Smith,[8] attention has been called to the importance of a species of intestinal worm as a factor in the transmission of blackhead in turkeys. Since the present investigation has been chiefly confined to the study of the inoculated disease, no conclusive evidence has been obtained relative to this interesting observation.

With the production of the disease by inoculation, we now have a means of studying more carefully the properties of the virus, its resistance to variation of temperature, to drying, to chemicals, etc. It is now possible to ascertain the effects on the parasite of various drugs administered to the infected host, and to attempt to modify the virus. It may also prove possible to demonstrate the virus in birds suspected of being carriers.

SUMMARY AND CONCLUSIONS

A distinct form of blackhead may be produced in turkeys by the subcutaneous inoculation of liver lesions from acute cases, and this disease may be propagated apparently indefinitely, by subinoculation into normal turkeys. This form of the disease is characterized by the appearance of a primary local lesion which first shows appreciable development from 5 to 7 days after inoculation; by the occurrence of secondary lesions regularly in the lungs, rarely in the liver and kidney, and by the later involvement of the liver and kidney (tertiary lesions) through the dissemination of the parasite from the involved lungs. In addition to weakness, loss of appetite, and sulphur colored feces—symptoms which are seen in the spontaneous disease—there is coughing and more or less dyspnea. The inoculated disease has been invariably fatal.

The incubation period is commonly 11 days, but varies between 11 and 17 days. The appearance of symptoms evidently indicates sufficient involvement of vital organs to interfere seriously with function. The rapid development of the subcutaneous lesion is not attended either by loss of weight or by symptoms, neither of which appears until after internal organs are involved.

In the course of their migration through the tissues from the site of inoculation, some of the parasites penetrate the veins and are

[8] Jour. Exper. Med. 1920, 31, p. 647.

carried to the lungs where they for the most part lodge and produce lesions. The disease thus metastasizes by way of the blood stream in a manner similar to that of certain tumors. The distribution of the parasites is thus governed by their ability to penetrate vessels, and by their size and physical properties which cause them to lodge in capillaries.

The expansion and contraction of the lungs evidently serve to dislodge organisms so that these organs are not as effective filters as the liver.

The parasites develop readily in a variety of tissues and organs: mucous membranes, connective tissues, both smooth and striated muscle, lung, liver and kidney. Macroscopic lesions of the kidney and microscopic lesions of the lung have been found in spontaneous blackhead.

The inoculation of chickens has resulted negatively except in newly hatched chicks in which self-limited local lesions, and in one instance, secondary lesions in the lungs, were produced.

A certain proportion of pigeons have proved susceptible to the extent of developing transient self-limited local lesions. Positive results were obtained in 30% of those inoculated. The lesions are essentially similar to the subcutaneous lesions of the turkey, but after developing actively from the fifth to the eighth day undergo regression. Rabbits, guinea-pigs and mice have proved nonsusceptible.

Blackhead may be contracted spontaneously from acute cases of its inoculated form, probably from the ingestion of food or water contaminated by discharges from the respiratory tract.

An attempt to transmit the disease through the agency of a species of "blow fly" has failed, but it is possible that this or related species may play a part in the dissemination of the disease.

Exposure of a young turkey to common fowls, after a long period of isolation, has been followed by the contraction of blackhead—unless the infection is to be attributed to the "blackhead-fed" flies ingested by it 37 days previously.

Neither the administration of tartar emetic nor of quinin has served to check the course of the infection.

Blackhead may be produced by the introduction of organisms beneath the skin, and is not dependent on the invasion of the tissues by myriads of flagellates that have multiplied in the lumen of the gut.

No flagellates have appeared in the ceca of newly hatched chickens following the ingestion of large amounts of blackhead virus derived from subcutaneous and lung lesions.

Cases of spontaneous blackhead occur in which there are no demonstrable flagellates in the cecal contents or in the gland lumina.

The definite transmission of the disease from one turkey to another by inoculation at once removes the great uncertainty which has attended all experiments in the past which have had to depend on the natural and not well understood method of transmission by the exposure of healthy turkeys to supposedly infected turkeys.

The demonstration of the uniform susceptibility of the normal turkey throughout its period of growth, not only opens the way for further investigation of the question of transmission, but also serves as a basis for future work on other problems relating to the virus of blackhead.